Application of urea dosing for alkalinity supply during anaerobic digestion of vinasse

ABSTRACT

Pushed by demand for renewable energy, the ethanol industry in Brazil is expanding. However, production of 1 m$^3$ of ethanol generates around 13 m$^3$ of liquid residues (vinasse), so this expansion results in an increasing need for a more adequate destination of these residues. Nowadays the vinasse is dispersed on the sugar cane fields in the practice of fertirrigation, but anaerobic digestion of this residue may be a better solution, additionally offering an alternative source of energy, able to complement hydroelectric power supply in the dry season. However, when trying to digest vinasse at reduced hydraulic retention times, complications arise from its strong tendency toward acidiﬁcation, upsetting the fragile balance of transformations normally occurring under anaerobic conditions. For successful operation of an anaerobic treatment process with acceptable hydraulic residence times, increasing alkalinity levels inside the reactor is necessary. In the present work we show that pH regulation by means of urea dosing, in spite of the risk posed by ammonia toxicity towards methanogenic biomass, can be a viable alternative to avoid vinasse acidiﬁcation. The ammonia formed in urea conversion remains in solution, rather than escaping to the biogas, and so its use as fertiliser can offset its cost of application in the process.

Key words | bicarbonate, pH control, UASB, urea, vinasse

INTRODUCTION

The last century saw great progress for humanity, especially as a result of large-scale availability of cheap energy from fossil fuels. However, due to environmental concerns and foreseeable imbalances between supply and demand of oil, ethanol (alcohol) is gaining worldwide importance as an automotive fuel. This is especially the case in Brazil, where a program of incentives for the use of ethanol has been in place since 1970 (De Almeida et al. 2007; Ribeiro & de Abreu 2008). Ethanol, in this case, is produced from sugar cane, where the sugary syrup obtained from milling the plants is fermented and then distilled to produce hydrated and anhydrous ethanol. Apart from producing ethanol, this process inevitably results in production of about 13 m$^3$ of waste (vinasse, or ‘vinhaça’ in Portuguese) per m$^3$ of ethanol (van Haandel 2005). This residue is, until now, disposed almost entirely by application on the cane fields, a practice known as ‘fertirrigation’, as it returns the mineral and nutrient content to the fields, and can considerably improve yields while reducing fertiliser use (de Resende et al. 2006). Unfortunately, problems are caused by the amounts of organic matter and acidity of the material. At the same time a significant part of energy content of the sugar cane is wasted. Given the sharp increase in installed distillation capacity since 2006, an increasing necessity to improve these practices has become obvious.

Current practice can be improved by subjecting the vinasse to anaerobic digestion prior to fertirrigation: after anaerobic digestion the nutrients will still be available, but organic matter and acidity will be drastically reduced. This process will also permit co-generation of electricity from the produced biogas in the dry season, when it can be a welcome addition to hydro-electric power generation, now responsible for 83% of electric power generation in Brazil (BP 2010).

An obstacle for successful anaerobic digestion of vinasse though, is its composition. Due to a considerable sugars content, low initial pH (around 4) and the total absence of alkalinity, vinasse is subject to rapid acidification, where methanogenesis rather than hydrolysis of organic matter is rate limiting. When no countermeasures are taken, the pH can easily reach values as low as 3.2, impeding the
methanogens from converting the intermediate organic acids into methane gas. As a result, a strategy to control process pH or alkalinity is needed, as simply feeding vinasse to an anaerobic reactor without such a strategy will lead to either process instability or the process running only at very low organic loading rates (OLRs) (when effectively the feeding rate, rather than the methane production rate, becomes the rate limiting step). Examples of strategies for control of process pH, other than applying a very large HRT, are: (i) dosing of a base like NaOH, (ii) dosing of buffering compounds like NaHCO₃ (Harada et al. 1996; Siqueira et al. 2008), (iii) effluent recycling, and (iv) automated process control. In this last case, the main advantage is that the process can run at a pH closer to the lowest pH possible, as countermeasures can be taken immediately when conditions might become adversary (Spanjers & van Lier 2006).

Keeping in mind the application of the treated vinasse for fertirrigation, in 2005 Adrianus van Haandel suggested studying urea dosage (van Haandel 2005). Enzymatic hydrolysis of urea (CO(NH₂)₂) produces CO₂ and ammonia, resulting in addition of 2 eq. of alkalinity per mol of urea when the process pH is below 6.5. The release of these compounds results in an increased buffering capacity of the system and may increase its pH, so that urea dosing can potentially improve anaerobic degradation of fast acidifying residues. On the other hand, ammonia is potentially toxic to anaerobic microorganisms (Sterling Jr. et al. 2001), an effect for which especially free ammonia (NH₃) is responsible (Hafner & Bisogni Jr 2009; Siles et al. 2010). Thus, too high a dosage should be avoided, especially when the pH rises above 7.5. However, at moderate to low pH all ammonia formed will be converted into the less toxic ammonium ion (NH₄⁺), assuring its presence in the liquid phase for fertirrigation and avoiding its loss to the biogas by stripping effects. Until now, no more literature describing urea application for this purpose has been found, so the main objective of this work was to study the effect of urea dosing on pH stabilisation, process stability and process efficiency, by means of batch experiments and by using a small-scale upflow anaerobic sludge blanket (UASB) reactor.

**MATERIALS AND METHODS**

**Batch experiments**

Batch experiments were carried out to verify the capacity of urea to provide alkalinity, and to compare this capacity to the buffering capacity of sodium bicarbonate. The experiments were carried out in sealed 500 mL bottles (with 400 mL liquid volume and 100 mL headspace). The liquid phase consisted either of diluted vinasse, collected at a nearby sugar factory/alcohol distillery, and characterised in an earlier paper (Formagini et al. 2010), or of a standard solution composed of 80% mixture of sugars (13% glucose and 87% sucrose [saccharose]) and 20% acetate (percentages relative to chemical oxygen demand (COD) concentration), using a COD of around 16 gO₂ L⁻¹. This standard solution is used as a model system for tests with pH stabilisation during anaerobic degradation, as due to its high content of simple sugars it is subject to fast acidification, like vinasse, while lacking the complex and variable matrix.

The effect of sodium bicarbonate (NaHCO₃) buffering was tested using concentrations of 0 (blank), 0.4 and 0.8 gNaHCO₃/gCOD, both with the standard solution and with vinasse, adjusted to an initial pH of 7.0. After the experiments with bicarbonate, buffering by means of different concentrations of urea was tested in the same way, using 0.215, 0.43 and 0.86 gurea/gCOD (supplying, in the first two cases, amounts of alkalinity roughly equivalent to the amounts supplied by the amounts of bicarbonate used), in all cases using duplicates with either vinasse or the standard solution. Initial pH was corrected only in part of the bottles, in order to verify the potential increase in pH resulting from alkalinity addition, as specified in Table 1.

A second set of batch experiments, intended to verify possible ammonia toxicity, was carried out in 3.25 L bottles,
filled with 2.50 L of diluted vinasse (14 ± 0.73 gCod L⁻¹) to which varying amounts of urea and phosphate buffer (0.1 M, pH = 7.00) were added. Conditions of these experiments are presented in Table 2.

In both series of batch experiments, nutrients were added according to literature (Chernicharo 2007). The biomass (4 gVSS L⁻¹) used originated from the UASB reactor treating effluent of the local FEMSA Coca Cola bottling plant. A mixture of 30% CO₂ and 70% N₂ (White Martins, Campo Grande, BR) was used as headspace in case the liquid phase contained bicarbonate as a buffer. In the remaining situations, pure nitrogen gas (White Martins, Campo Grande, BR) was used.

After closing, the bottles were incubated at a temperature of 30 ± 3 °C, and agitated twice a day. In the small bottles, biogas formation was measured using the displacement method (using 16% NaOH to absorb CO₂ from the biogas), whilst daily, by means of a syringe a small volume was withdrawn for pH determination. In the large bottles, biogas formation was measured using the same gas displacement method, but additionally a larger range of parameters was determined: pH (4500-H²O⁻), ammonia-nitrogen (4500-NH₃⁻-B), COD (5220-C) according to the corresponding methodology from Standard Methods for the Examination of Water and Wastewater (American Public Health Association 2005). The steady state condition of the reactor was assessed by observing COD removal (constant at around 95%) and by observing stability of the pH after each change in operational conditions (buffer dosage).

**Table 2** | Set-up of experiment in large (3.25 L) bottles for determination of urea/ammonia toxicity

<table>
<thead>
<tr>
<th>Code</th>
<th>Substrate</th>
<th>COD (mg COD L⁻¹)</th>
<th>pH initial</th>
<th>Urea (mM)</th>
<th>Additional buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-A</td>
<td>Vinasse</td>
<td>13.7</td>
<td>4.3</td>
<td>8.3</td>
<td>0.1 M KH₂PO₄/NaOH</td>
</tr>
<tr>
<td>1-B</td>
<td>Vinasse</td>
<td>14.7</td>
<td>7.0</td>
<td>8.3</td>
<td>0.1 M KH₂PO₄/NaOH</td>
</tr>
<tr>
<td>2-A</td>
<td>Vinasse</td>
<td>13.7</td>
<td>4.3</td>
<td>33.3</td>
<td>0.1 M KH₂PO₄/NaOH</td>
</tr>
<tr>
<td>2-B</td>
<td>Vinasse</td>
<td>14.7</td>
<td>7.0</td>
<td>33.3</td>
<td>0.1 M KH₂PO₄/NaOH</td>
</tr>
<tr>
<td>3-A</td>
<td>Vinasse</td>
<td>15.7</td>
<td>4.3</td>
<td>66.7</td>
<td>0.1 M KH₂PO₄/NaOH</td>
</tr>
<tr>
<td>3-B</td>
<td>Vinasse</td>
<td>14.7</td>
<td>7.0</td>
<td>66.7</td>
<td>0.1 M KH₂PO₄/NaOH</td>
</tr>
</tbody>
</table>

Continuous experiment

Continuous experiments were carried out in a 0.92 L glass UASB reactor, equipped with peristaltic pumps (Dosamini 5900, Provitec, São Paulo-SP, BR) for influent and recirculation, kept in a thermostated compartment at a temperature of 37 ± 0.7 °C. Biogas produced was monitored by means of a tipping-cell type gas flow meter (manufactured in-house), while the amount of CO₂ in the biogas was monitored using an infrared CO₂ sensor (D01, Madur, Vienna, AT). Temperature and pH (PH1000, Provitec, São Paulo-SP, BR) were monitored as well. All monitoring was performed on-line as described in earlier work (Boncz et al. 2008).

The reactor was inoculated with 300 g of sludge (with a volatile suspended solids (VSS) content of 0.085 gVSS/gsludge) from a 40 L UASB reactor treating vinasse. The reactor was fed with vinasse diluted to a concentration of 8 gCOD L⁻¹, and flow was adjusted for a hydraulic retention time (HRT) of 2 d, keeping the OLR unchanged during the experiment. Effluent recirculation of 0.41 L d⁻¹ effectively doubled upflow velocity. Buffering was done initially by dosing NaHCO₃ (0.8g L⁻¹, over time reduced to 0.15 g L⁻¹), then by dosing both NaHCO₃ and urea (at 0.12 g L⁻¹), and finally using urea only, increasing its dosing to 0.15 g L⁻¹ and later 0.2 g L⁻¹. At specific intervals effluent samples were taken and analysed for total alkalinity, total acidity, ammonia (4500-NH₃-B) and COD (filtered) (5220-C) according to the corresponding methodology from Standard Methods for the Examination of Water and Wastewater (American Public Health Association 2005). The steady state condition of the reactor was assessed by observing COD removal (constant at around 95%) and by observing stability of the pH after each change in operational conditions (buffer dosage).

**Simulations**

Batch experiments were simulated using Simnon 3.0 for Windows (SSPA Systems, Gothenburg, SE), using a simplified model taking into account: COD conversion by SO₄⁻² reduction, COD conversion by hydrolysis of sugars into acetic acid and subsequent transformation of acetic acid into CO₂ and CH₄, and urea hydrolysis, as well as VFA, sulphide, ammonia, phosphate and inorganic carbon acid/base equilibria and gas/liquid phase partitioning. The resulting pH was calculated forcing a balance of charge neutrality, the initial pH being set by defining initial concentrations
of a generic metal cation $M^+$ (implying presence of the infinitely strong base $MOH$) or a generic anion $X^-$ (implying presence of the infinitely strong acid $HX$). Equations used in the model are given below:

$$
CO_2(g) = CO_2(aq) + H_2O = H_2CO_3 = H^+ + HCO_3^-
\Rightarrow 2H^+ + CO_3^{2-}
$$  
\[(1)\]

$$
H_3PO_4 = H^+ + H_2PO_4^- = 2H^+ + HPO_4^{2-} = 3H^+ + PO_4^{3-}
$$  
\[(2)\]

$$
H_2S(g) = H_2S = H^+ + HS^- = 2H^+ + S^{2-}
$$  
\[(3)\]

$$
NH_3(g) = NH_3(aq) + H_2O = NH_4^+ + OH^-
$$  
\[(4)\]

$$
H_2O = H^+ + OH^-
$$  
\[(5)\]

$$
CH_4(g) = CH_4(aq)
$$  
\[(6)\]

The distribution of the species involved in the (multi-step) equilibria (Equations (1–7)) was resolved algebraically, using acidity and basicity constants as cited in the Handbook of Chemistry and Physics (Weast 1995), and Henry’s constants as cited by Sander (1999), at a temperature of 35 $^\circC$, whilst changes in species concentrations from transformations (Equations (8–15)) were solved by numerical integration. Kinetic constants $k_{so}$ (rate of sulphate oxidation), $k_{uh}$ (rate of urea hydrolysis) and $k_{ma}$ (methano-genic activity) were estimated fitting experimental results.

### RESULTS AND DISCUSSION

#### Batch experiments

To counter the acidification occurring during anaerobic digestion of vinasse, various authors have used bicarbonate addition in the past, dosing between 1 and 10 $g_{\text{NaHCO}_3}/g_{\text{COD}}$ (Harada et al. 1996; Siqueira et al. 2008). Results from the first batch, not presented here, showed that a concentration of around 0.6 $g_{\text{NaHCO}_3}/g_{\text{COD}}$ (200 mM) in case of vinasse with 16 $g_{\text{COD}}$ L$^{-1}$ should be sufficient.

The effect of urea is even stronger, as can be seen in Figure 1: without prior neutralisation even the lowest concentration applied (0.215 $g_{\text{urea}}/g_{\text{COD}}$, 57 mM in this case) succeeds in elevating the pH to 6.5 within 4 d, when vinasse is being digested. Only the solution of sugars and acetate shows, initially, acidification at a rate higher than the rate of urea hydrolysis, so that even in the initially neutralised situation, and when dosing considerable amounts of urea, some acidification occurs, until ammonia production from urea catches up. Modelling the system shows comparable results (Figure 2); to neutralise the acidity produced by acidification of sugars in vinasse, without the presence of any buffering compounds, even small doses of urea (17 mM) should be sufficient to maintain pH at least above the initial value, whereas larger doses of urea show the possibility of rapidly elevating pH to values around 9. It can also be seen that, even without any additional measures, conversion of COD and subsequent stripping of CO$_2$ should be sufficient to obtain a neutral effluent. However, in practical applications the long HRT needed to obtain a stable process in this way would be unworkable. Increasing concentrations of urea increase the CO$_2$ content of the biogas, lowering its specific heat of combustion, one more reason (apart from possible ammonia toxicity) to limit urea dosing to exactly the amount needed for pH stabilisation.
To study in more detail what happens during digestion of vinasse in the presence of urea, under conditions as simulated above, batch experiments were performed on a bigger scale, permitting withdrawal of samples for analysis of composition of the liquid. The results are presented in Figures 3 and 4, and are slightly different from those obtained by modelling, as the simple model does not take inhibition of methanogenesis in case of low pH values into account.

Acidiﬁcation occurs under all conditions, but when the pH is raised above 6.5, conversion of the intermediate organic acids into CO₂ and methane will elevate the pH to a final value above 7.0, causing removal of dissolved COD and a corresponding production of biogas. Ammonia produced from the urea remains in solution as NH₄⁺. However, when dosing 4 g urea L⁻¹, the rapid hydrolysis combined with the slightly increased pH cause a short spike of free ammonia in solution on day 2, which may be the reason for the slightly slower rate of COD conversion in this case when compared with the situation in which only 2 g urea L⁻¹ was dosed. In slight disagreement with predicted results, when 2 g urea L⁻¹ is dosed without additional phosphate buffering, the pH drops to around 5.5 and methanogenesis occurs at a much slower rate, hindering methanogenesis and biogas production, and impeding neutralisation of the system (Figure 4).
Continuous experiments

Mixed results were obtained with continuous operation of the UASB treating vinasse. Initial operation, with application of sodium bicarbonate as buffer, showed good results, with a stable pH of 6.7 ± 0.2 independent of the amount of NaHCO₃ dosed (Figure 5). Reduction of bicarbonate dosing, with dosing of an additional 0.12 g urea L⁻¹, shows a continuation of stable operation, albeit at slightly lower pH (6.5 ± 0.5). Alkalinity slightly reduces over time, with acidity remaining stable, causing a slight increase of the Acidity/Alkalinity ratio from an initial 0.15 up to 0.24 on day 17, while soluble COD removal remains constant at 96%. However, 3 d after stopping bicarbonate dosing on day 15, switching to dosing only 0.15 g L⁻¹ of urea, first biogas production and then the pH dropped considerably. Finally, after 20 d of operation, the pH settled at a value of 4.5, in spite of increasing urea dosing even more, to 0.2 gurea L⁻¹.

It should be remembered that in continuous operation much smaller doses of urea are necessary than in batch experiments, as in continuous operation the production of alkalinity occurs simultaneous to acidification, rather than after this process, and much less buildup of acidic metabolic intermediates occurs. Given the drop in pH, obviously a higher amount of urea dosing than applied here seems necessary to stabilise the process. But the cause of the sudden decrease of methanogenic activity, followed by the sudden drop of pH (rather than a drop of pH preceding the loss of methanogenic activity) cannot have been caused by ammonia toxicity (as was suspected in an earlier experiment (Formagin et al. 2010)). Ammonia toxicity occurs only above levels of several grams per litre (Garcia & Angenent 2009; Siles et al. 2010), depending on process pH (and thus free NH₃ concentration) and temperature (applying a higher operating temperature may mask the toxic effect of ammonia on the biomass) (Garcia &
Angenent 2009), but the maximum concentration of ammonia found in the reactor was only 96 mg L$^{-1}$ on day 20 and the process pH never exceeded 7.0. One possible explanation for the loss of methanogenic activity is that H$_2$/CO$_2$ is an important intermediate in the degradation of vinasse. We could observe that biogas production stopped 2 d (1 HRT) after bicarbonate deprivation of the reactor, and before any change in the pH occurred. It thus seems that bicarbonate is used as an exogenous source of carbon, to convert H$_2$ into CH$_4$, analogous to what is observed in methanol degradation (Paulo et al. 2003). Previous experiments with a reactor running under similar conditions (and during a much greater span of time), but with a different seed sludge (Formagini et al. 2010), now support this hypothesis. In that case, after 72 d of experiment, an interruption of biogas production was also observed a few days after bicarbonate deprivation. However, this event was concomitant with a high dosage of urea (4 g/L) and before any change in the pH occurred. It thus seems that bicarbonate was disregarded, as ammonia toxicity was believed to be the cause for the interruption, even more because the pH in that experiment did not fall below 5.8. But in that experiment, after 12 d bicarbonate was dosed again, and 2 d later biogas production started to recover.

CONCLUSIONS

Anaerobic digestion of vinasse in a simple UASB is possible and can help reduce environmental problems commonly associated with the practice of fertirrigation, as well as contributing to diversification of the energy matrix in Brazil. Rapid acidification, followed by inhibition of methane production, a common problem with anaerobic digestion of vinasse, can be avoided by increasing alkalinity in the bioreactor, by dosing urea, and by bicarbonate or phosphate buffering. Dosing of urea (0.25 gurea/gCOD in batch digestion or 0.015 gurea/gCOD, complemented with bicarbonate dosing, in a continuous system) can improve the efficiency of the anaerobic degradation of the organic matter present in the vinasse. Care must be taken to avoid overdosing though, as ammonia toxicity can interfere with methane production. Maintaining the pH of the process below 7.24, almost all (>99%) of the ammonia will be retained in the effluent in the form of ammonium salts that will be dispersed on the fields during fertirrigation. The costs of urea and phosphate dosing should, under such circumstances, be offset by the reduced costs of application of fertiliser.

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